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Optimization of albumin extraction from carp (*Cyprinus carpio Linnaeus*, 1758) under conditions of varying temperature and HCl solvent concentration

Nurfaidah[1*] & Ahmad Fahrizal[2]
[1]Department of Fishery Product Technology, Faculty of Fisheries and Marine Science, Mulawarman University, Indonesia
[2]Department of Fisheries Resources Management, Faculty of Fisheries, Muhammadiyah Sorong University, Indonesia
[*]corresponding author: nurfaidah@fpik.unmul.ac.id

ABSTRACT

Albumin, a globular protein, plays a crucial role in various biological functions, including regulating blood osmotic pressure and transporting small molecules in the body. Given the increasing demand and the substantial production costs associated with albumin derived from human plasma, it is imperative to explore alternative sources, such as fish. The objective of this study is to optimize the extraction of albumin from common carp (*Cyprinus carpio*) using hydrochloric acid (HCl) as a solvent at varying concentrations and heating temperatures. The present study employed a factorial experimental design, incorporating two variables: The initial concentration of HCl was measured to be within the range of 0.05–0.2 M, and the heating temperature was adjusted between 27 and 60°C. The dependent variable in this study was albumin yield percentage. The fish meat was then homogenized with hydrochloric acid at a ratio of 1:2, subsequently heated in a water bath for a duration of 30 minutes, filtered, dried, and weighed. The yield data were subjected to analysis using two-way ANOVA and Tukey's HSD test (SPSS 22). The results demonstrated that albumin yields ranged from 0.94% to 3.55% (w/w). The highest yield was achieved with 0.1 M HCl at 35°C, reaching 3.55%, while the lowest yield was obtained with 0.2 M HCl at 27°C, which was 0.94%. Moderate temperatures and the appropriate HCl concentration allow effective protein denaturation without excessive damage, making it the optimal extraction method. These findings suggest that the optimal condition for maximizing albumin yield from common carp is extraction with 0.1 M HCl at 35°C. The study also provides a foundation for further exploration of alternative extraction methods and environmentally friendly solvents, as well as an economic feasibility assessment at the industrial scale.

Keywords: extraction method; fish albumin; protein denaturation

INTRODUCTION

Albumin is a globular protein that plays a crucial role in various biological functions, including regulating blood osmotic pressure and transporting small molecules within the body's circulation. In practical applications, albumin is widely used in the medical field as a plasma expander for patients with severe burns or hypovolemia, and is also formulated into injectable drugs and nutritional supplements. In the food and pharmaceutical industries, fish-derived albumin is increasingly being developed into functional food products and dietary supplements aimed at improving nutritional intake and aiding recovery in patients with low protein levels (Belinskaia et al., 2021). The demand for albumin is increasing due to population growth

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and the expansion of the pharmaceutical and nutritional industries. The global albumin market was valued at USD 6.35 billion in 2023 and is expected to grow from USD 6.57 billion in 2024 to USD 10.19 billion by 2032 (Fortune Business Insights, 2024). Currently, most commercially available albumin is derived from human blood plasma (The Lancet Haematology, 2017), which poses high risks and has relatively expensive production costs (Soedjanaatmadja et al., 2021). Therefore, exploring alternative sources of albumin has become important, with fish being one such alternative.

Fish are recognized as a potential source of protein, including albumin, and are available in large quantities and easily accessible. Additionally, fish represent a relatively inexpensive resource, making them a more economical option for albumin production. Fish albumin is being explored in functional foods, such as fish cereal (Fitriyani & Nuraenah, 2023). Nurfaidah et al. (2021) explored albumin content in various freshwater fish species and found that common carp, snakehead, and catfish are highly promising as albumin sources. Based on this data, this study utilizes common carp (*Cyprinus carpio* Linnaeus, 1758) as the source of albumin for maximizing extraction recovery. Various methods have been employed for extracting water-soluble proteins, including steaming, vacuum drying, and freeze-drying with different solvents (Andreeva, 2022; Asfar, Tawali, Abdullah, & Mahendradatta, 2014; Firlianty, 2016; Hariati, Yuniarti, Endariani, Kusuma, & Wiadnya, 2019; Tri Paus Hasiholan Hutapea et al., 2023; Nugroho, 2013; Romadhoni, Afrianto, Pratama, & Grandiosa, 2016; Santoso, 2019; Wiranata et al., 2015).

The extraction process of albumin from fish requires optimization to achieve maximum yield, both in terms of quantity and quality. A crucial step in this process is optimizing the heating temperature and adjusting the concentration of the solvent, as both factors significantly influence the efficiency of albumin extraction. Variations in heating temperature can affect the structure and solubility of proteins, including albumin (Andoyo, Diani, & F, 2023). High temperatures may lead to protein denaturation, while low temperatures might not be effective enough to separate albumin from other tissue components in the fish. According to Wirahadikusumah (1981), protein structures begin to deteriorate and denature at temperatures above 40°C. Albumin, being a water-soluble protein, experiences reduced solubility at temperatures between 50-60°C (Foegeding et al., 1986). Extraction at temperatures above 60°C can result in significant precipitation and produce a cloudy white extract due to the coagulation of plasma proteins caused by heating.

In addition to temperature, the presence of a solvent plays a vital role in disrupting tissue matrices and altering the solubility of proteins during extraction. Acidic solvents such as hydrochloric acid (HCl) can denature proteins and shift their

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solubility profile by modifying the pH of the environment. This condition promotes protein unfolding and facilitates the separation of albumin from other tissue components. Therefore, the use of HCl is essential in enhancing the efficiency of albumin extraction from fish tissues. A combination of optimal heating temperature and the appropriate concentration of HCl is expected to maximize albumin extraction yields. Romadhoni et al. (2016) stated that the quality of albumin is influenced by the extraction method used. The choice of solvent in albumin extraction significantly affects the yield obtained. Previous studies have shown that HCl was more effective than water or NaCl as a solvent in extracting albumin from fish, with 0.1 M HCl producing the highest protein extract from snakehead fish meat (Asfar et al., 2014; Romadhoni et al., 2016). Previous studies on albumin extraction from fish using HCl have also been conducted on snakehead and catfish (Asikin & Kusumaningrum, 2018; Jamaluddin, Hasnawati, Yuyun, Pitriani, & Widodo, 2021; Setiawan, Intanon, Chummitri, Sringarm, & Sathanawongs, 2024).

This study aims to optimize the albumin extraction process from fish through variations in heating temperature and HCl concentration. Through this optimization, it is hoped that the albumin produced can meet industrial needs with lower production costs and fewer risks compared to albumin derived from human blood plasma. This research also contributes to diversifying functional protein sources that are vital in the food and pharmaceutical industries, while simultaneously supporting the utilization of abundant fish resources.

METHODOLOGY

This study investigated common carp (*Cyprinus carpio*). The materials used included HCl solutions at various concentrations (0.05 M to 0.2 M), and distilled water. The equipment used consisted of a laboratory homogenizer, 250 mL glass beakers, a shaking water bath (Memmert WNB 7 or equivalent), vacuum filtration apparatus, an analytical balance, drying oven (set at 55°C), and HDPE storage bags. The fish samples analyzed were live specimens obtained from wet markets around Makassar. The collected fish ranged in size from 340 to 824 g. All fish were transported alive to the laboratory, where they were immediately euthanized using thermal shock at ±10°C in a bucket containing water and crushed ice. The fish, weighing approximately 3 kg, were divided into three groups of equal weight as replicates. The fish samples were then scaled, gutted, deboned, and filleted, followed by thorough washing with clean running tap water and left to drain. The fish meat was then minced, pre-homogenized in a commercial blender, placed in high-density polyethylene (HDPE) zip-lock bags, and stored at -20°C until further analysis.

After thawing the pre-homogenized fish meat at room temperature, 50 g of the meat was precisely weighed into a 250 mL glass beaker, diluted with

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HCl at a solvent-to-meat ratio of 1:2, and homogenized for 1 minute using a laboratory homogenizer. The homogenized sample was then incubated at 50°C for 1 hour in a continuously shaking water bath. Following incubation, the sample was filtered under reduced pressure, and the volume of the filtrate was measured. The albumin extract was dried in an oven and weighed to determine the dry weight (Nurfaidah, et al., 2021). Table 1 presents the treatment conditions used to maximize the recovery of fish albumin.

Table 1.
Treatment of Solvent Concentration and Heating Temperature

Concentration of HCl	Temperature
0.05 M	27°C
0.075 M	30°C
0.1 M	35°C
0.125 M	40°C
0.15 M	45°C
0.175 M	50°C
0.2 M	55°C
0.2 M	60°C

This experimental study was conducted with three replications. The temperature of 27°C was selected to represent room temperature conditions, serving as a control to observe baseline extraction efficiency without additional heating. This provides a reference point to compare the effect of elevated temperatures (30°C–60°C) on albumin yield. Yield was calculated by weighing the final dried extract. The liquid albumin extract was weighed before being dried in an oven at 55°C for 72 hours. After drying, the sample was reweighed and compared to the initial weight. The formula used to calculate the albumin yield is as below:

$$Yield\ (\%) = \frac{Final\ weight}{Initial\ weight} \times 100\%$$

Data Analysis

The data obtained from this study were analyzed using two-way analysis of variance (ANOVA) to examine the main effects and interactions between the two independent variables under investigation. The analysis was conducted using SPSS 22 software. Prior to performing the ANOVA, assumptions of normality, homogeneity of variances, and independence of observations were tested to ensure the reliability of the results. A significance level of 0.05 ($\alpha = 0.05$) was used to determine statistical significance. When the ANOVA indicated significant effects, post-hoc comparisons were performed using Tukey's HSD test to identify specific differences between the observed parameters, with a confidence level of 95%.

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Given that the primary focus of the study was on the yield of albumin, the analysis was tailored to assess the differences in albumin yield between the groups. The results are presented with an emphasis on the statistical significance of these differences, ensuring that the findings are robust and meaningful. All statistical procedures were conducted to maintain rigor and ensure the validity of the conclusions drawn from the data.

RESULTS AND DISCUSSION

The results of the albumin yield measurements from common carp using different solvent concentrations and heating temperatures can be seen in Table 2.

Table 2.
Percentage Yield of Common Carp Albumin Extract

[HCl]	Temperature (°C)							
	27	30	35	40	45	50	55	60
0.050	1.47±0.10	1.78±0.12	2.64±0.15[a]	2.03±0.08	2.03±0.09	1.98±0.11	3.14±0.13[b]	1.89±0.14
0.075	2.10±0.12	2.33±0.14	2.27±0.16[b]	2.85±0.13	2.84±0.12	1.74±0.10	2.66±0.15	1.77±0.11
0.100	2.18±0.13	2.33±0.14	3.55±0.16	2.15±0.12	2.44±0.15	2.42±0.14	2.36±0.13	1.66±0.11
0.125	1.48±0.10	1.71±0.11	2.21±0.14	1.89±0.09	1.93±0.12	2.13±0.10	1.95±0.08	1.43±0.11
0.150	1.29±0.09	1.95±0.12	1.95±0.10	2.00±0.11	2.22±0.13	1.86±0.10	1.960.12	2.15±0.14
0.175	1.14±0.08	1.40±0.09	1.85±0.11	1.44±0.10	1.58±0.10	1.63±0.10	1.74±0.11	1.63±0.09
0.200	0.94±0.07	1.14±0.08	2.05±0.13	1.13±0.09	2.34±0.15	1.64±0.10	1.55±0.11	1.64±0.12

Notes: [a]Significant differences from other conditions, particularly: 0.050 M HCl at 40°C, 0.100 M HCl at 60°C, and 0.125 M HCl at 55°C. [b]Significant differences from other conditions, notably: 0.050 M HCl at 27°C, 0.100 M HCl at 60°C, and 0.175 M HCl at 27°C.

Figure 1 illustrates the percentage yield of albumin extracted from common carp (*Cyprinus carpio*) across varying temperatures and HCl concentrations. The data reveal that the yield is significantly influenced by both the temperature and the concentration of HCl, with the highest yield observed at 35°C and 0.1 M HCl as shown by the results of two-way ANOVA which indicated statistically significant main effects ($p < 0.05$) for both variables. The highest yield was observed at 35°C and 0.1 M HCl, confirming this combination as the optimal condition. This trend indicates the optimal conditions for maximizing albumin extraction, where moderate heating and precise HCl concentration synergistically enhance the efficiency of the process.

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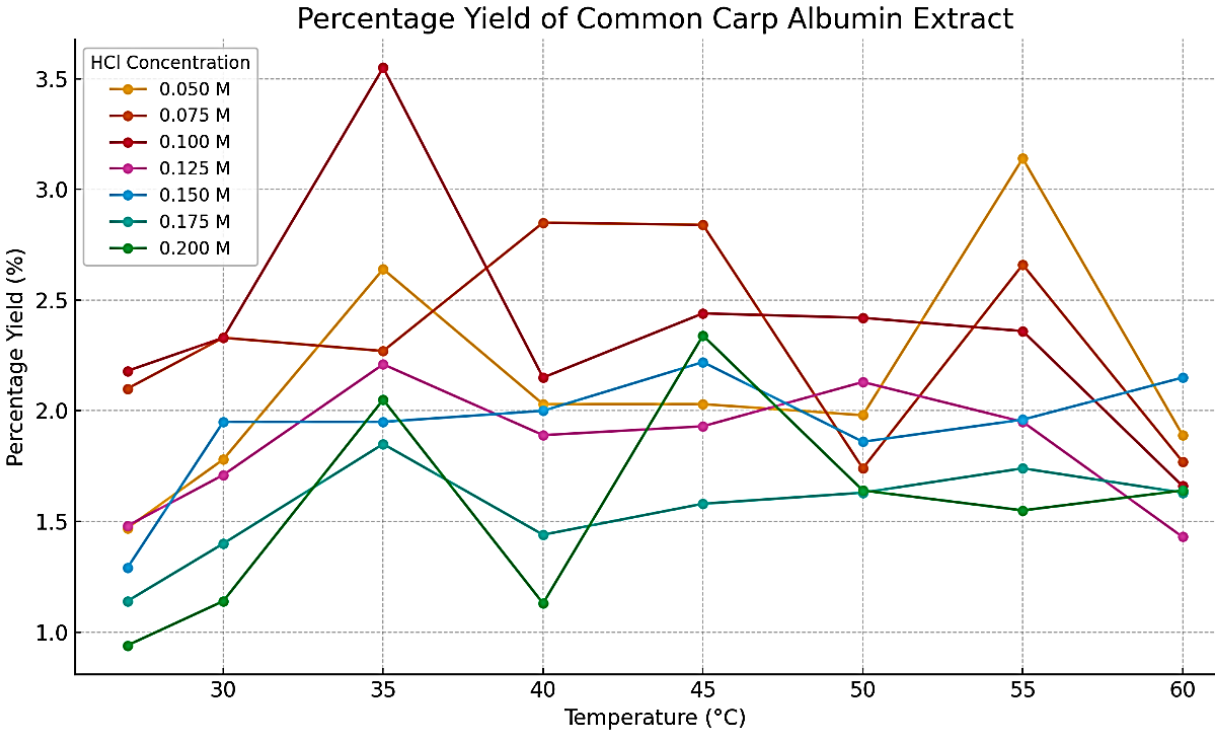


Figure 1. Percentage yield of common carp albumin extract at varying temperatures (27°C to 60°C) and HCl concentrations (0.05 M to 0.2 M). The graph highlights the optimal extraction condition of 35°C with 0.1 M HCl, yielding the highest percentage of albumin

Yield refers to the percentage or final weight of the extract compared to the initial weight of the material or sample observed. This study yielded albumin in powder form. Albumin yield was measured to assess the effectiveness and efficiency of the extraction method used (Qixing et al., 2014). In general, 0.1 M HCl at 35°C produced the highest albumin yield, indicating this combination as the optimal condition for albumin extraction from fish. This can be explained by the adjustment of pH towards the isoelectric point of albumin, where protein solubility is at its lowest, leading to maximum precipitation. Heating at temperatures above 60°C tends to reduce albumin yield, indicating excessive denaturation that decreases protein solubility in the solvent. Moderate heating temperatures (around 35°C-45°C) proved effective in increasing yield, particularly at HCl concentrations corresponding to the isoelectric point of albumin (around 4.6).

Effectiveness of Boiling Method in Albumin Extraction

The extraction method used in this study is the boiling method using a waterbath with various temperature and solvent concentration treatments to yield more albumin compared to other extraction methods. Although this study did not compare other methods, previous studies can be referenced in determining the best extraction method (Chasanah et al.,2019; Susilowati et al., 2016). These studies showed that the boiling

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extraction method produced higher yields than steaming and vacuum drying. Other studies also showed similar results, where boiling with solvents yielded albumin with yields of 5.83-7.65% (Romadhoni et al., 2016), compared to 1.77% with steaming (Nugroho, 2013) and 4.71% with vacuum drying (Hariati et al., 2019). This can be attributed to the fact that boiling not only increases the solubility of albumin in water but also facilitates a crucial denaturation process. This denaturation leads to the breakdown of cell membranes and muscle fibers, which in turn increases the efficiency of albumin release from the fish tissue matrix into the solvent (Vujadinović et al., 2014). This process allows albumin, which is inherently prone to denaturation, to dissolve more easily, thereby increasing the yield of the extract obtained. In other words, the ability of boiling to reduce the intramolecular strength of albumin through denaturation makes it a highly effective extraction method, resulting in higher albumin yields than other methods that do not utilize intensive heating.

The boiling method is widely acknowledged for its effectiveness in boosting albumin yield while being more cost-effective and easier to implement on an industrial scale compared to techniques like vacuum drying. Numerous studies have emphasized the benefits of boiling, particularly in terms of heat transfer efficiency and nutrient preservation (Rashidi, Hormozi, & Mohsen, 2020).

Boiling serves as a highly efficient mechanism for heat transfer, facilitating significant thermal energy transfer even at low temperature differences, which is advantageous for industrial use. Compared to more complex and costly techniques such as freeze drying or vacuum drying, which are commonly used in the pharmaceutical and food industries for high-purity protein isolation, boiling offers a simpler and more cost-effective option—especially for medium-scale applications where extremely high purity is not required. While vacuum drying can produce purer protein fractions, it typically requires 4–5 times more energy input and significantly longer processing time (Meena et al., 2022), making boiling more practical for pilot-scale or initial-stage extraction operations.

Beyond increasing albumin yield, boiling also enhances the nutrient content of food products. For example, boiling fish for 15 minutes greatly lowers harmful phosphorus levels without affecting protein quality (Tsai, Chiu, Wu, & Sun, 2021). The straightforwardness of boiling makes it more practical for industrial applications, in contrast to the complexity and higher costs associated with vacuum drying (Pioro, 2024).

Effect of Solvent Concentration and Heating Temperature on Albumin Yield

In general, the heating temperature significantly affects the yield of albumin produced. At all tested HCl concentrations, increasing the temperature from 27°C to 35°C resulted in a higher albumin yield. For instance, at an HCl concentration of 0.1 M, the yield increased from 2.18% at 27°C to 3.55% at 35°C, which was the highest yield observed in this

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study. After reaching 35°C, further temperature increases tended to decrease the albumin yield at most HCl concentrations, as seen in the yield decline at 50°C and 60°C.

HCl concentration also plays a crucial role in determining the albumin yield. Lower HCl concentrations, such as 0.05 M, generally resulted in lower yields at the same temperature, with the highest yield being only 3.14% at 55°C. In contrast, at higher HCl concentrations (e.g., 0.175 M and 0.2 M), the albumin yield tended to be lower across all tested temperatures, indicating that excessively high HCl concentrations may not be effective in separating albumin from fish tissue.

The highest albumin yield suggests that the combination of conditions is the most effective in separating and extracting albumin from fish tissue compared to the other tested conditions, based on statistical analysis showing that this combination produced a significant difference in albumin yield ($p<0.05$). Conversely, the lowest yield of 0.94% was observed with a solvent HCl concentration of 0.2 M and a temperature of 27°C (without heating). When compared to previous studies using HCl as a solvent, the yield range observed in this study is within a similar order of magnitude. However, it is important to note that those studies used different fish species, such as snakehead and catfish, which have varying protein compositions and albumin content. Therefore, direct comparisons should be interpreted cautiously and not used to conclude the superiority of one method over another (Romadhoni et al., 2016; Asikin & Kusumaningrum, 2018) in snakehead fish albumin, as well as 0.156% in catfish (Jamaluddin et al., 2021). The interaction between various solvent concentration and heating temperature variations resulted in diverse albumin yield weights. However, the quantity of fish albumin yield is influenced not only by the extraction method and solvent used but also by the environment in which the fish lives. Susilowati et al., (2015) found that the chemical composition of protein extract from wild and cultivated snakehead fish showed that albumin levels in wild snakehead fish were higher than in cultivated ones. The chemical composition of fish also varies based on its intrinsic characteristics (Suwandi & Winem, 2014).

In this study, the temperature of 35°C and 0.1 M HCl concentration worked synergistically to maximize the albumin yield. The moderate temperature aided in the proper protein denaturation process, while the optimal HCl concentration ensured that the denatured protein could precipitate efficiently (Opdensteinen et al., 2021). Albumin solubility was optimally enhanced, while at the same time, these conditions supported the efficient precipitation of albumin after the denaturation process, resulting in the highest yield in the extraction process. The temperature of 35°C caused sufficient denaturation of albumin to release it from the fish tissue matrix without causing excessive structural damage. This controlled denaturation opened up the protein structure, allowing albumin molecules to dissolve into the solvent. The temperature of 35°C was also low

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enough to avoid excessive denaturation that could lead to protein aggregation, reducing albumin solubility. Temperatures higher than 35°C tended to increase this risk, thereby reducing the effectiveness of the extraction. Studies indicate that heating at 50-70°C enhances albumin recovery, with specific protocols showing maximum extraction at 63-67°C (T. P.H. Hutapea, Suprapto, & Kurniawan, 2022; Setyawati & Putra, 2024).

Prolonged heating can intensify protein denaturation, which decreases solubility and compromises the quality of the extract. This underscores the importance of closely monitoring both time and temperature during the extraction process (Setyawati & Putra, 2024).

Safety and Effectiveness of HCl as a Solvent

HCl at a concentration of 0.1 M was able to lower the pH of the solution close to the isoelectric point of albumin, which is around pH 4.6 (Asfar et al., 2019). At the isoelectric point, proteins like albumin have the lowest solubility and tend to precipitate. This decrease in solubility allows albumin to be more easily separated from the solvent after the extraction process. The 0.1 M HCl concentration is strong enough to cause a significant pH drop but still within a safe range to maintain the integrity of the albumin structure. This concentration allows the release of albumin from the fish tissue without causing excessive protein degradation.

CONCLUSION

The findings of this study demonstrate that the extraction of albumin from carp using HCl as a solvent can maximize albumin yield. The highest yield was achieved at an HCl concentration of 0.1 M and a temperature of 35°C, yielding 3.55%. The combination of a 35°C temperature and 0.1 M HCl concentration functions synergistically to optimize the extraction process by effectively denaturing proteins without causing excessive structural damage. Increasing the temperature beyond 35°C has been observed to reduce albumin yield due to the risk of excessive denaturation. Furthermore, higher HCl concentrations have been shown to decrease the effectiveness of the extraction process. These findings indicate the necessity for additional studies to investigate a broader range of extraction conditions, the utilization of more environmentally friendly alternative solvents, and an economic feasibility assessment of this method for industrial applications.

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